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RELATIONSHIPS OF PLANT ROOTS TO THE LESION NEMATODE, PRATYLENCHUS PENETRANS (COBB, 1917) FILIPJEV & SCHUURMANS STEKHOVEN, 1941.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

I INTRODUCTION

There is disagreement in the nematological literature as to the method of plant-parasitic nematode accumulation around plant roots. Many investigators have suggested that a chemical stimulus emitted from plant roots causes a taxis or kinesis resulting in nematode accumulation at the roots. Others have advanced the idea that root finding by nematodes is due to random movement, accumulation being the result of the nematodes remaining near the root surface once they have contacted it.

A variety of factors have complicated the question. Many different genera and species of both plants and nematodes have been used in tests. Little concern has been given to the rate of migration of the nematodes used as test organisms. A confusion has arisen from lack of uniformity and specificity of the terms "attraction" and "accumulation".

Pratylenchus penetrans, the lesion nematode, is an endoparasite whose host range has not yet been completely determined. It causes economic loss to crops by stunting the plants and wounding the roots so that other pathogens may enter.

Despite its obvious pathogenicity, P. penetrans has not been extensively studied, and migration experiments with this species are practically non-existant. One of the reasons for this has been the difficulty of culturing large numbers of experimental animals. Recent culture methods have overcome this problem, however.

The attempt of this investigation was to study nematode relations with plant seedling roots under conditions such that attraction may be

differentiated from accumulation, using various hosts in two kinds of media. In addition, the areas of the roots producing attraction and the root areas penetrated by nematodes were investigated.

II REVIEW OF LITERATURE

The definition of the word "attraction" is of particular importance in any analysis of previous investigations. Many investigators, both early and recent, have, in measuring attraction, used indices such as number of nematodes in roots, number of galls produced on roots, and number of nematodes reaching the plant side of a volume of medium in 24 or 48 hours. Such indices are misleading unless one takes into account factors such as failure of penetration of roots, nematode migration from roots, and speed of movement of the nematode in a particular medium.

There is fairly complete agreement in the literature that accumulation of nematodes around living roots readily occurs. Early investigators (Stewart 1921, Steiner 1925) concluded that some sort of root exudate present in varying concentrations around plant roots attracted the nematodes to the root surface. These conclusions, however, were based on the fact that accumulation occurred. Steiner (1925) put forward the hypothesis that a chemotaxis was responsible and that the amphids, sensory structures located in the anterior end of the nematode, were chemoreceptors which detected chemical plant root exudates some distance from the root. This hypothesis has been accepted or restated by most workers (Dropkin 1955, Christie 1960). Kühn (1959), Weischer (1959), and others have proposed the possibility that random movement is responsible for root finding. Wallace (1960) having examined additional works points out that the function of the amphids is still unknown.

An early report was made by Linford (1939) who tested the attraction

of various plant tissues in a special medium of pulverized volcanic ash, and another of quartz sand. The former allowed direct observation of nematode activity. Accumulation of Heterodera marioni (Meloidogyne sp.)[1] larvae to the growing point of tomato seedling roots was apparent by direct observation in 2 - 3 hours and often in 1 hour after inoculation with nematodes. This he termed attraction. In soil results were less decisive. Fresh tissue from green leaves and stems of diverse plants, and also yeast colonies in agar, were attractive. Cooked tissue was attractive after decomposition set in. Wounds made by single nematodes penetrating the roots seemed to bring about mass invations and fresh wounds made by other means appeared attractive since several larvae entered these holes.

Linford concluded that attraction did occur and that migration was chiefly along roots, the amount of migration depending on the intertwining of roots. The nature and distance over which attraction is effective was not defined. The evidence that Pratylenchus pratensis and Rotylenchus spp. fed in the piliferous zone rather than in the zone of elongation suggested that two attractants may be operating. Also, root knot larvae were attracted by non-hosts. He indicated that this was a failure by the nematode to distinguish a suitable host by attractant alone.

Gadd and Loos (1941) studied nematode response to roots using Anguillulina pratensis (Pratylenchus pratensis). They measured attraction by the percent of nematodes in the roots after 24 and 48 hours in proportion to the number inoculated. Greater percentage of attraction occurred when the seedlings were in the sand more than 24 hours prior to

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[1] See appendix B for scientific naming and synonomy.

inoculation with nematodes. That growing roots with tops were more attractive suggests that the attractive substance was a product of growth. Fewer nematodes entered excised roots, and dead roots killed by boiling water were only slightly attractive. That decaying roots were unattractive disagrees with Linford's findings. Emigration of nematodes from roots was observed in some cases, but no explanation for this was given.

Wieser (1955, 1956) demonstrated attraction and repulsion of Meloidogyne hapla larvae to germinating seedlings and excised roots of various plants in quartz sand over a 24 hour period. If a high percentage of nematodes were in the half of the dish containing roots, these roots were considered attractive. Roots were judged repellent if the percentage in the root side of the dish was considerably below 50%. Test materials were placed 5 mm from the side where egg masses were introduced. Sterile conditions were not maintained. The apical 2 mm of tomato seedling roots were repellent, the following 6 mm attractive, and behind this zone neutral or slightly repellent conditions existed. Other host seedlings were repellent or variable in the way nematodes reacted to them. His conclusion was that chemical attraction occurs, but that an "antagonism" between the attractive agent in living roots and a repellent produced upon death and decay of roots was responsible for variations in attractiveness. The great variability in Wieser's results suggests that perhaps the total sum of his results is a neutral effect, the extremes, attraction and repulsion, being the result of normal sampling error. Also, no mention is made of the attractiveness of cut ends of roots, although Linford found that Meloidogyne sp. were attracted to them in sand and volcanic ash.

Widdowson, Doncaster, and Fenwick (1958), using roots in nutrient

culture found that the highest concentration of Heterodera rostochiensis larvae were in areas behind the root tips or at the junction of main and lateral branches of excised roots. They concluded that perhaps attractant substances produced in these regions caused this phenomenon. These findings with H. rostochiensis agree with Wieser's, who worked with M. hapla.

Race and Henderson (1959) noted penetration of mature root zones, and occasional penetration of tips of various plant roots by Pratylenchus penetrans.

Kühn (1959) pointed out that many experiments regarding attraction were actually measuring accumulation of nematodes at plant roots which they had found by moving at random in the test medium. In discussing the work of others, he emphasizes that the time lapse between inoculation of the media with nematodes and the recording of numbers near plant roots was sufficient in many cases to allow nematodes to search the entire medium. No attraction was detected to potato eye pieces using H. rostochiensis larvae whose rate of movement had been determined. His results, which showed accumulation, were identical with theoretical values calculated using random movement as a basis.

No oriented movement was found by Weischer (1959) and random movement was described as a chemokinesis. The results agreed basically with Kühn's in that movement of H. schactii Schmidt and H. rostochiensis larvae was random through sand. Areas extremely close to the root surface, however, were attractive, but the main effect of root exudations was stimulatory - causing faster random movement and enhancing the chances of root findings, factors which Kühn failed to comment upon.

Working with Pratylenchus brachyurus and P. zeae, Endo (1959) reported that no migration from the site of inoculation occurred unless plant roots were in the soil. Horizontal migration to corn roots was farther in Norfolk sandy loam than in Portsmouth loam or in Cecil clay loam. Endo concluded that factors conducive to greater nematode motility, such as aeration, pore size, and particle size, were optimum in the sandy loam. That host roots were conducive to nematode migration is in agreement with Linford's conclusion that migration along roots occurs.

Bergmann and Van Duuren (1959a) testing rape-seedling roots and root diffusates of rape-seedling and sugarbeet for attractiveness concluded that movement of H. schachtii larvae was random over moist filter paper, and agar strips, or in quartz sand. Later (1959b) they stated that certain bacterial colonies in association with plant roots produced attractants or repellents to the larvae while other colonies were toxic to them. They concluded that bacteria in the plant rhizosphere may play an important role in attraction of nematodes to plant roots. This was an effect overlooked by many workers.

Working mainly with Heterodera spp. Wallace (1956-61) found that nematode movement in sand was greatly affected by pore size, depth of the water film in the pores, and presence of host roots. Nematodes moved from an area of low moisture content to one of higher moisture content in a block of sand. Tomato seedlings planted in the drier end of the block reversed this movement, the longer the seedling roots were in the sand prior to inoculation of the block with nematodes, the greater the degree of orientation toward the seedling. Observations twice daily of larvae moving over glass beads 200 μ in diameter (the same size as sand in which

optimum movement occurred) in the presence of plant roots failed to show random movement. The larvae were attracted to roots 2 cm away. H. schachtii larvae accumulated in sand in an area of a sintered glass funnel where cress seedlings had previously been growing. This suggests that the attracting substance was chemical in nature. Wallace concluded that a chemical attractant was responsible for root finding over a distance of 2 cm.

Attraction to growing root pieces in test tubes containing a thin film of 0.5% nutrient agar was demonstrated by Peacock (1959) using M. incognita larvae. At the end of 24 hours accumulation to a defined area immediately behind the apical meristem of tomato was noted. Mass invasion through a small puncture, as Linford had noted by Meloidogyne sp., was evident. No repellent areas of root were found, which is in disagreement with Wieser's findings with M. hapla, but cessation of linear growth of the roots resulted in cessation of attraction. Larvae migrated and remained near a cellophane barrier in an attempt to reach the root tip. Using activated charcoal in a sand medium Peacock (1961) found that gall formation by M. incognita larvae was prevented. His conclusions were that chemical attraction does exist, that the attractant substance is able to diffuse through a cellophane barrier, and that it is capable of being absorbed on particles of activated charcoal. No explanation is given, however, as to why the larvae did not find the roots by random movement. This suggests that the charcoal did more than just absorb the attractant.

Bird (1959) found that areas of low redox potential along tomato roots in agar were most attractive to M. javanica and M. hapla larvae. The root zone of elongation corresponded to the area of lowest redox potential.

Measuring a way from this area either into the agar or up and down the root, he found that the redox potential became more positive creating a concentration gradient against which nematodes were attracted.

Working with M. hapla, Lownsberry and Viglierchio (1960) concluded that accumulation around tomato seedlings was a composite result of random movement, attractants from roots and nematode detention in the root zone. M. hapla larvae accumulated at a dialysis membrane interposed between the larvae and the seedling on the surface of the sand medium. Greater attraction occurred when the membrane was absent. The small pores of membrane (24Å) indicated that the attractive material was of low molecular weight. Bacteria were present in all tests.

Viglierchio (1961) studied attraction of M. hapla, M. incognita acrita and Heterodera schachtii to host plant roots in sand. Accumulation occurred at a membrane separating roots from the nematodes in 48 hours. The attracting stimulus was effective over a distance of at least 10 mm.

Certain investigators in attempts to determine the cause of nematodes finding roots have used specific compounds or special physical and chemical conditions. The first to use redox potential as an explanation was Bird (1959). He found that strong reducing agents such as sodium dithionite and colonies of the aerobic bacterium Escherichia coli (Migula) were capable of lowering the redox potential in agar. Both were attractive to root knot nematode larvae. These results, supported by his finding that the attractive z one of elongation of tomato roots had the lowest redox potential, led him to conclude that this physical condition was responsible for nematodes' finding plant roots. In a later work Bird (1960) modified this view somewhat by concluding that probably a combination of factors

which affect various nematode species differently was responsible. Among these factors were CO_2 concentration, redox potential, and glutamic acid. Klingler (1961) found attraction of Ditylenchus dipsaci to sodium dithionite, but potassium permanganate, a strong oxidizing agent was also attractive. Removal of redox potentials by buffers did not prevent attraction of nematodes to CO_2.

Klingler (1959, 1961) performed investigations with CO_2 which he found that D. dipsaci was attracted to CO_2 emanating from a fine cannula in agar. Attraction occurred after the agar medium was buffered to remove pH and redox potential gradients.

After studying respiration rates of various nematodes in atmospheres of different CO_2 concentrations, Rohde (1960) suggests that high concentrations of CO_2 which may build up in the vicinity of plant roots act as orthokinetic substances. Such substances decrease nematode activity by depressing their respiration and causing nematode accumulation near roots.

Peacock (1961), however, using ion exchange resins, found that even though the resins removed any CO_2 emanating from tomato roots in sand, invasion and gall formation still occurred.

Galvanotoxis was exhibited by Panagrellus redivivus (L.) as found by Caveness and Panzer (1960). Movement to the cathode at 0.02 ma (milliampers) and above in 1% water agar and sandy loam soil was noted for this species and several other genera including Pratylenchus sp.

Jones (1960) reported that the vinegar eelworm (Turbatrix aceti) aggregated around the cathode when an electric current was passed through vinegar and distilled water in Petri dishes. The potential gradient rather than size of the current appeared to elicit the response.

H. schachtii larvae moved toward the anode in a sand-tap water system, while D. dipsaci moved toward the cathode. In distilled water reversal of direction of movement of D. dipsaci was noted when poles were reversed, movement being always to the cathode. Klingler (1961), however, found no attraction of D. dipsaci to either the anode or the cathode when an electric current was applied to agar through a simple platinum or copper wire electrode. This apparent contradiction suggests that perhaps the electric current modifies the medium in some way.

Various compounds were tested for attraction in agar by Bird (1959). Gibberellic acid 0.1%, B-indolylacetic acid 0.1M, digitoxin 0.01%, oubaine, and tryptophane 0.001M all proved unattractive. No attraction to various pH buffers, O_2 or CO_2 concentrations from 1-20% in bicarbonate buffers was noted.

In order to analyze the foregoing literature one must keep several factors in mind. (1) The various investigators have used a variety of genera and species of both nematodes and plants in attempts to explain movement of nematodes to plant roots. (2) In very few investigations has the rate of movement of the nematodes used in the test been calculated, and in even fewer cases has the element of time in relation to speed of movement and volume of medium in which the nematode and plant root are placed been considered. (3) Definitions of the terms "attraction" and "accumulation" have been neither uniform nor specific in many cases.

Plant parasitic nematodes appear to be stimulated by several means. Diffusable attractants from root areas do exist and are effective over measurable distances. The specific analysis of these attractants has not yet been determined. Root exudates, bacterial colonies and exudates,

and CO_2 are probable contributing factors. Galvanotaxis and areas of low redox potential may affect nematode movement, but are factors needing additional qualification.

Random movement probably occurs under conditions where stimuli such as those above are not effective. Until more is learned of the chemical and physical environment of nematodes in soil and the distance over which attractants are effective, the importance of random movement versus oriented attraction will remain a question. According to Wallace (1961) effects of nature such as gravity, light, and heat either do not affect plant nematode migration or their effect is unknown.

The area of root most attractive to nematodes appears to depend on the particular genera or species. Parasites exhibiting a rather highly specialized type of parasitism such as root knot (Meloidogyne spp.) seem to prefer the root zone of elongation, while Pratylenchus, which may be an exception, appear to be attracted and to feed on nearly any portion of the root. Definitive statements on attractive areas await further investigations involving many different nematodes.

III OBJECTIVES

The purpose of this investigation is to critically study the relationships between host plant roots and nematodes migrating in various media under conditions such that distinctions may be made between oriented attraction, kineses, and normal random movement. The selection of three host plants (carrot, alfalfa, and tomato) to be tested for attraction is based on the premise that externally each presents a mature root system that is basically different anatomically. (It was later found that this criterion was not valid as in the seedling stages used, these differences were slight.) Pratylenchus penetrans, the lesion nematode, has been selected as a test organism because of its omnivorous habit and unspecialized form. This nematode is an endoparasite attacking, feeding, and reproducing inside the roots of a wide range of plants. In addition to being a pathogen itself, causing lesions, necrosis, and death of roots, it prompts invasion by other disease organisms which may enter the feeding wounds. Large numbers of larvae and adults may be found migrating through the soil surrounding infested roots presumably in search of a fresh food supply.

The factors to be considered in this investigation are the influences of the medium through which migration is occurring (sand and agar), host plant species, areas of roots most attractive to the nematode and point of entry, distance of nematode from host root, decapitated seedlings and excised roots of host, and exudates from host roots.

IV PROCEDURES

A. Culturing nematodes:

Pratylenchus penetrans was cultured axenically on alfalfa callus tissue following the technique of Krusberg (1960). Sterile alfalfa seedlings were grown on nutrient agar slants in 25 mm diameter test tubes. The addition of 2, 4-dichlorophenoxyacetic acid to the agar stimulated the production of soft, fleshy, root callus tissue. Surface sterilized P. penetrans specimens added to the callus culture reproduced rapidly, and within 6 weeks to 2 months several thousand nematodes were produced from a small amount of inoculum.

For each experiment, animals were obtained by removing the agar and the plant from the tube, placing both in a Baerman funnel and collecting the nematodes in a small amount of water. This operation seldom took longer than four hours. Axenic conditions were not maintained after removal of the nematodes from the tubes.

B. Obtaining test seedlings:

Test seedlings were obtained by germinating seeds on one per cent water agar in Petri dishes. Dupuits alfalfa (Medicago sativa L.)seeds were scarified and surface sterilized by placing them in concentrated H_2SO_4 for 15 minutes and rinsing with sterile distilled water. Richmeat tomato (Lycopersicon esculentum Mill.), Gold Pak carrot (Daucus carota L.), seeds were surface treated for 15 minutes in sodium hypochlorite solution prepared by mixing Chlorox bleach with an equal volume of water. Alfalfa seedlings were transplanted in migration chambers after 1 to 3 days and

carrot and tomato seedlings were transplanted in 4 to 8 days. In order to obtain marigold seedlings for preliminary experiments involving determination of root areas to which nematode accumulation occurred, Flame marigold (Tagetes patula L.) seeds were treated with sodium hypochlorite solution as above and transplanted in chambers after 7 days.

C. Method of measuring movement in agar:

Measurement of nematode movement was conducted in a rectangular chamber containing agar in which a seedling had been placed at one end, the other end remaining undisturbed. Hereafter these ends will be referred to as plant and blank ends of the chamber (Fig. 1). Polystyrene chambers 46 x 22 x 5 mm were marked on the undersurface into two 14 mm end sections and an 18 mm center section and surface treated in Chlorox solution. Each chamber was then filled with one per cent water agar to a depth of 4-4.5 mm, and before gelation occurred a seedling was added to the approximate center of the plant end. Care was taken to insure that the root was well covered with agar and that the stem and leaves did not touch the agar.

Twenty-four hours later a 7 mm diameter cylinder of agar was removed from the center of the chamber. Approximately 60 nematodes were pipetted into this cavity in a small volume of water and cool but ungelled agar was pipetted into the cavity to the level of the surrounding agar, covering the nematodes. This formed, upon gelation, a continuous layer through which nematodes moved easily.

After three hours incubation at 26 - 27°C and 100 per cent relative humidity, the chambers were removed and the nematodes observed for movement from the site of inoculation with a dissecting microscope. The number of nematodes found beneath the agar surface on the plant side and

on the blank side were counted and recorded separately.

When counting was completed the chambers were returned to the incubator until the next time interval had passed. Modifications of the above procedure were used for other experiments involving decapitated seedlings and excised apical sections of mature plant roots (1-1.5 cm long) in place of the whole seedlings and where seedlings were removed just prior to inoculation.

The mature plants were maintained in greenhouse pots prior to root excision (alfalfa- 8 months, carrot- 5 months, and tomato- 6 months). Since other investigators (Linford, 1939) had found nematode accumulation at cut ends of roots, care was taken to prevent these areas from being exposed to the nematodes.

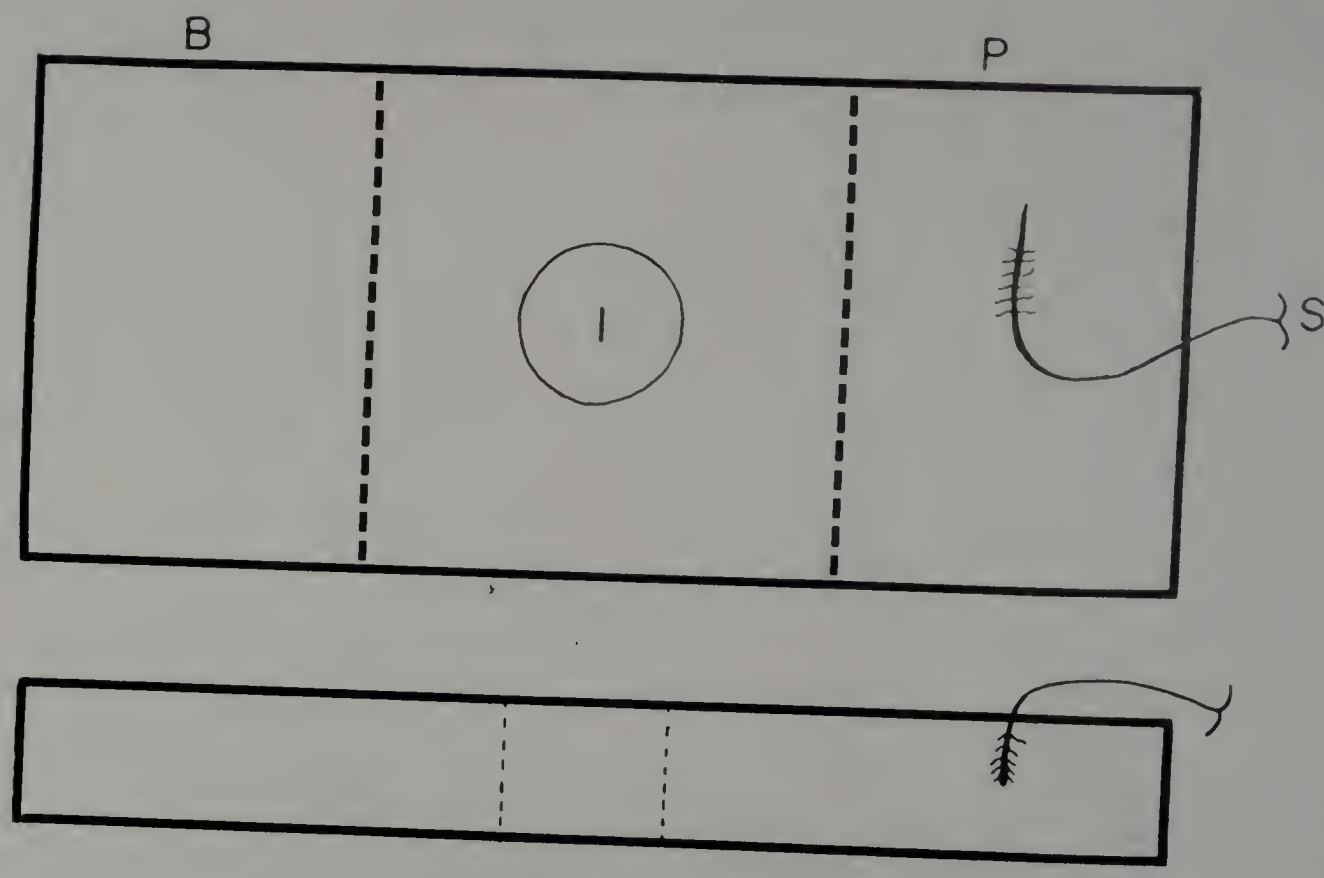
D. Calculating speed of nematode movement without seedling influence:

In order to obtain speed of nematode migration without the influence of a seedling, two methods were used. First, the method as described immediately above in C. was modified only insofar as the 3 chamber sections were subdivided into smaller ones and counts were made at shorter time intervals.

The second method consisted of filling glass tubes (10 cm long and 2 mm in diameter) with molten one per cent agar, allowing it to gell, and adding nematodes to one end. After time intervals of one and three hours the distance from the point of inoculation to the nematode which had migrated furthest away from the source was measured and speed in mm per hour was calculated. In both methods incubation temperature was 26 - 27°C.

Figure 1. Migration chamber used to measure movement of P. penetrans in agar. B-blank end, P-plant end, S-seedling, I-inoculation site.

Figure 2. Migration chamber used to measure movement of P. penetrans through sand. S-seedling, R-rubber band, PN-paper napkin, SN-sand, I-inoculation site.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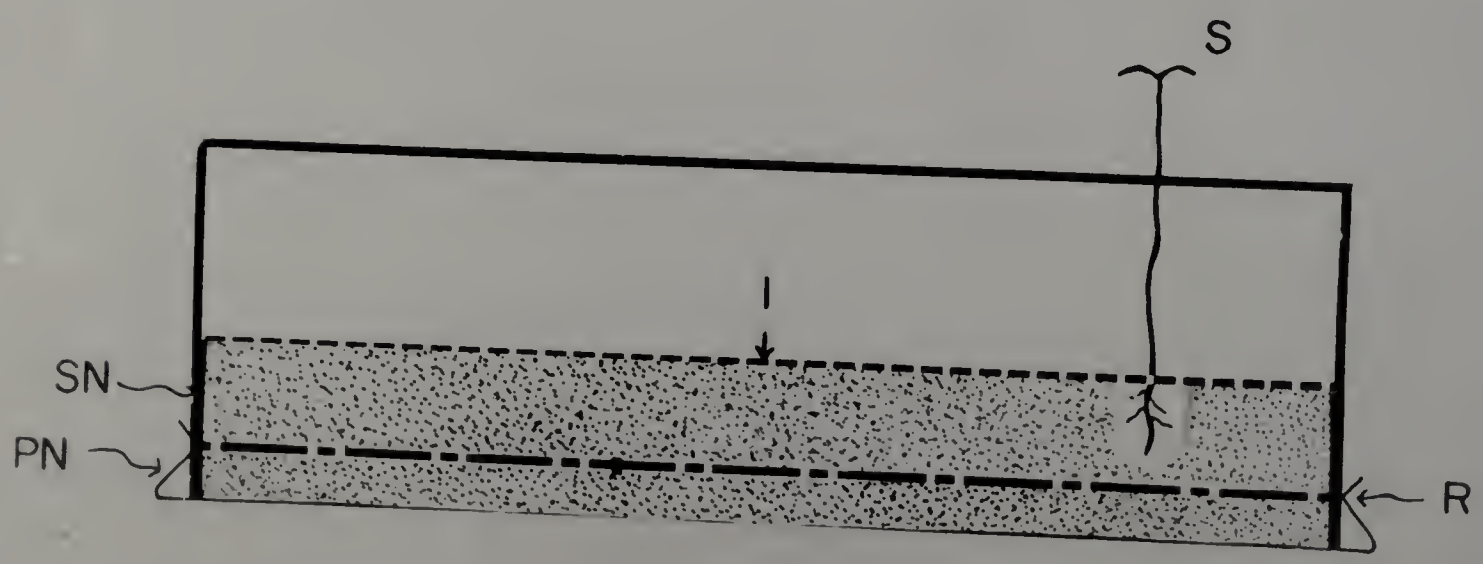
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E. Method of measuring movement in sand:

Measurement of movement in sand was achieved using rectangular plastic chambers with water porous bottoms (Fig. 2). Chambers were made by cutting out the bottoms of small plastic boxes 42 x 20 mm long and wide and 16 mm deep with a hot scalpel. Rectangular sections of paper napkin were stretched across the bottoms and fastened to the chambers by means of rubber bands. A 7 mm deep layer of quartz sand was then placed in the bottom of the chamber and brought to field capacity. This was accomplished by spreading 9 grams of sand in the bottom and wetting it evenly with 3 ml of water. This amount of water had been previously determined to be the quantity needed to bring the moisture content of the sand to field capacity. Seedlings of the test plants were transplanted at the center of one end in each of the chambers, the other end being undisturbed. As before, these ends will hereafter be referred to as plant and blank ends. All chambers were kept at 100 per cent relative humidity until inoculation. After 24 hours,75 nematodes were pipetted to the center of the sand in a small quantity of water. The Petri dishes containing the inoculated chambers were then placed in an incubator at 26 - 27°C.

After the interval of time required for migration, the sand in the chamber was divided into three sections in order to obtain counts of the nematodes. The sand was removed in a single block by cutting the rubber band and sliding the chamber up and away from the sand. A cut was then made 14 mm from each end through the sand and napkin. The sample and seedling, if present, was placed in a beaker to which tap water was added. The center section of sand was discarded.

The sand was washed three times and the wash water examined for

nematodes. The napkin and seedling were observed for nematodes, then discarded. Each beaker was swirled by hand to float the nematodes out of the sand, the wash water being poured into a marked syracuse dish, and the number of nematodes recorded.

When measuring speed of movement in sand without a seedling, a slight modification of the above technique was made. The sand was divided into five sections instead of three, consisting of a 14 mm center section and a 6 and 8 mm section on each side of this. The sand was washed and nematodes counted as above.

F. Measuring the distance over which attraction to tomato seedlings is effective in agar:

Measurement of the distance over which P. penetrans is attracted to tomato seedlings in agar was accomplished by basically the same method as described in C., except that longer chambers were used and plants were placed at greater distances from the inoculation site. Chamber dimensions were 154 mm long, 20 mm wide and 8 mm deep. Lines were drawn on the under-surface at 9 mm and 23 mm from the center in both directions. The area enclosed by the two outermost lines was approximately equal to the area of the chambers in C. These lines were used as guides for placing the seedlings and inoculating with nematodes. One per cent water agar was added to the chambers making a layer 5 mm deep. A tomato seedling was added to one end of each chamber at one of two positions, either 44.5 mm from the site of inoculation or 69.5 mm from this site. Twenty-four hours after placing the seedlings, 60 nematodes were pipetted into the center of the chamber as in C. Readings and recordings were made at 3 and 6 hours after inoculation and the number of nematodes in the plant

end was compared with the number in the blank end.

G. Measuring effect of tomato seedling diffusate in agar on nematode movement:

The effect of tomato seedling diffusate on nematode migration in agar was measured at 3 and 6 hours after inoculation of the chambers described in C. with P. penetrans. Diffusate was procured by germinating tomato seedlings for 10 days in sand placed in a sintered glass funnel. By means of suction, utilizing a side arm flask, the diffusate was drawn from the sand into the flask, poured into a vial, stoppered, and either used immediately or stored in a refrigerator at 9°C. The tomato seedling diffusate is defined as the liquid obtained by this suction method. This liquid presumably contained root products, chemical root emanations, bacteria and bacterial exudations, and other phyto-organisms and chemicals associated with the tomato seedling rhizosphere.

The same material (exudate) was used for all aspects of this test and concentrations were made by varying the number of drops applied to filter paper sections by means of a drying technique. Concentrations of 4, 12, 20, 28, and 36 drops were applied one at a time to #1 filter paper and each drop air dried with a fan before the next drop was applied. The circular area covered by each drop prior to drying amounted to 18 mm in diameter. Three 6.5 mm disks were cut from this area with a paper punch after the prescribed number of drops had been added. The plastic chambers were filled with a 5 mm layer of 1 per cent water agar and allowed to gell. The disks containing dried diffusate were inserted in the middle of end sections of the chambers so that the arc of the disk just touched the bottom of the plastic chamber and the largest surface area was exposed to

the site of inoculation. A control disk of untreated filter paper was added to the other end of each chamber. After 24 hours, 60 nematodes were pipetted into the center of the chamber as in C. above. Numbers of nematodes reaching the two ends were counted and recorded at the end of 6 hours after which the disks were removed and placed in dishes containing water to obtain nematodes which might have entered the paper. These individuals were counted and added to the numbers recorded for each end at 6 hours. A separate tally of the numbers of nematodes in the plant and blank disks was kept for a later comparison of this aspect of the test.

H. Statistical procedures:

The analysis of variance for two groups with equal replication was applied to the data obtained in testing for differences between blank and plant ends of the chambers. A method which considered unpaired observations and unequal variances involving computation of the $s_{\bar{d}}$ was used to test blank ends of control chambers against blank ends of chambers with seedlings. The least significant differences (L.S.D. .05) were computed wherever a significant difference between treatment means (blank and plant ends) was found. Significant values and L.S.D. figures are presented in the figure captions. Further explanation and examples of statistical procedures are located in Appendix A.

V ANALYSIS AND PRESENTATION OF DATA

In this investigation the term "attraction" was used if the following two criteria were met: (1) the mean number of nematodes that reached the plant end of a chamber was significantly greater (at the 5% level) than the mean number that reached the blank end; (2) this difference occurred at the critical time lapse after inoculation of the chambers with nematodes of 3 hours in the case of agar and 6 hours in sand. These time periods were selected after it was found that in the absence of seedlings the rate of migration of the fastest individual in agar was 10 mm per hour, while that in sand was 4-5 mm per hour. In similar control chambers equal numbers of nematodes moved to both ends of chambers (Table I). Based on these speeds of migration and considering the sizes of the two small chambers used, counts of the two (blank and plant) ends at time lapses greater than those above might give the appearance of attraction to the plant end, but would actually be "accumulation." Random movement could be responsible for this as the nematodes would have had a chance to search the blank end, migrate back through the site of inoculation, and enter the plant end.

It appeared by repeated observation that the larger, more active nematodes were the first observed in the root region. Smaller, less active individuals followed. It was with these "explorers", the first nematodes reaching the stimulus, that this investigation was concerned. In every experiment a large proportion of the nematodes either did not leave the inoculation site or moved out much more slowly than the explorers. Manipulation of the individuals inoculated may have caused this

TABLE I. P. penetrans migration in control chambers under no host seedling root influence at 3 hours in 1% water agar and in quartz sand at 3 and 12 hours after inoculation of chambers with nematodes. BL = blank ends of chambers.

Medium and Time	Treatment	I	II	III	IV	V	VI	VII	VIII	x	Log + one $\bar{x}$	.05 L.S.D.
AGAR	BL	2	1	3	0	4	5	0	0	1.9		
												n.s.
3 HR	BL	1	2	4	2	4	0	0	0	1.6		
SAND	BL	1	3	1	3	0	5	1	4	2.3		
												n.s.
3 HR	BL	0	0	0	5	1	2	0	1	1.1		
SAND	BL	10	3	3	8	31	1	2	6	8.0		
												n.s.
12 HR	BL	3	20	16	4	3	6	6	5	7.9		

adverse effect on motility.

A. Nematode migration to whole seedling roots in agar:

Data obtained by testing alfalfa, carrot, and tomato, hosts of P. penetrans, met the above criteria for attraction. Differences between plant and blank ends were highly significant (Fig. 3). The mean number of nematodes reaching the plant end continued to increase after 3 hours, while the mean number of nematodes going to the blank end remained fairly constant. The slight decrease in numbers counted in the plant end at 24 hours was the result of the nematodes entering the roots, thus being removed from the observed population. Penetration of roots actually was occurring at 12 hours, but continued nematode immigration to the plant end of the chambers more than offset this effect. Some of the individuals which entered the roots were later observed after staining the roots. The counts at time lapses greater than the initial one may have been the result of accumulation.

Ends of control chambers did not differ significantly from blank ends of host chambers in 3 hours (Fig. 3). This suggests that there was no chemokinesis operating as was found by Weischer (1959) in sand. It does not, however, rule out the possibility of this phenomenon existing in the plant ends of chambers in areas close to the roots.

B. Nematode migration to decapitated seedlings in agar:

Decapitated seedlings of the hosts tested were less attractive than roots with tops (Fig. 4). There was a highly significant difference between plant and blank ends of tomato chambers. A significant difference was noted for carrot, while alfalfa did not show an attractant effect (Table II). It was observed that linear growth of decapitated seedlings

Figure 3. Migration of P. penetrans in agar to seedling roots of alfalfa, carrot, and tomato and to blank ends of control chambers. Means based on at least 8 replications. Differences between plant and blank ends of chambers at the end of 3 hours were as follows: alfalfa chambers - **, L.S.D. .05 = .265; carrot chambers - **, L.S.D. .05 = .357; tomato chambers - **, L.S.D. .05 = .288. Differences between blank ends of control chambers and blank ends of seedling chambers at the end of 3 hours were not significant.

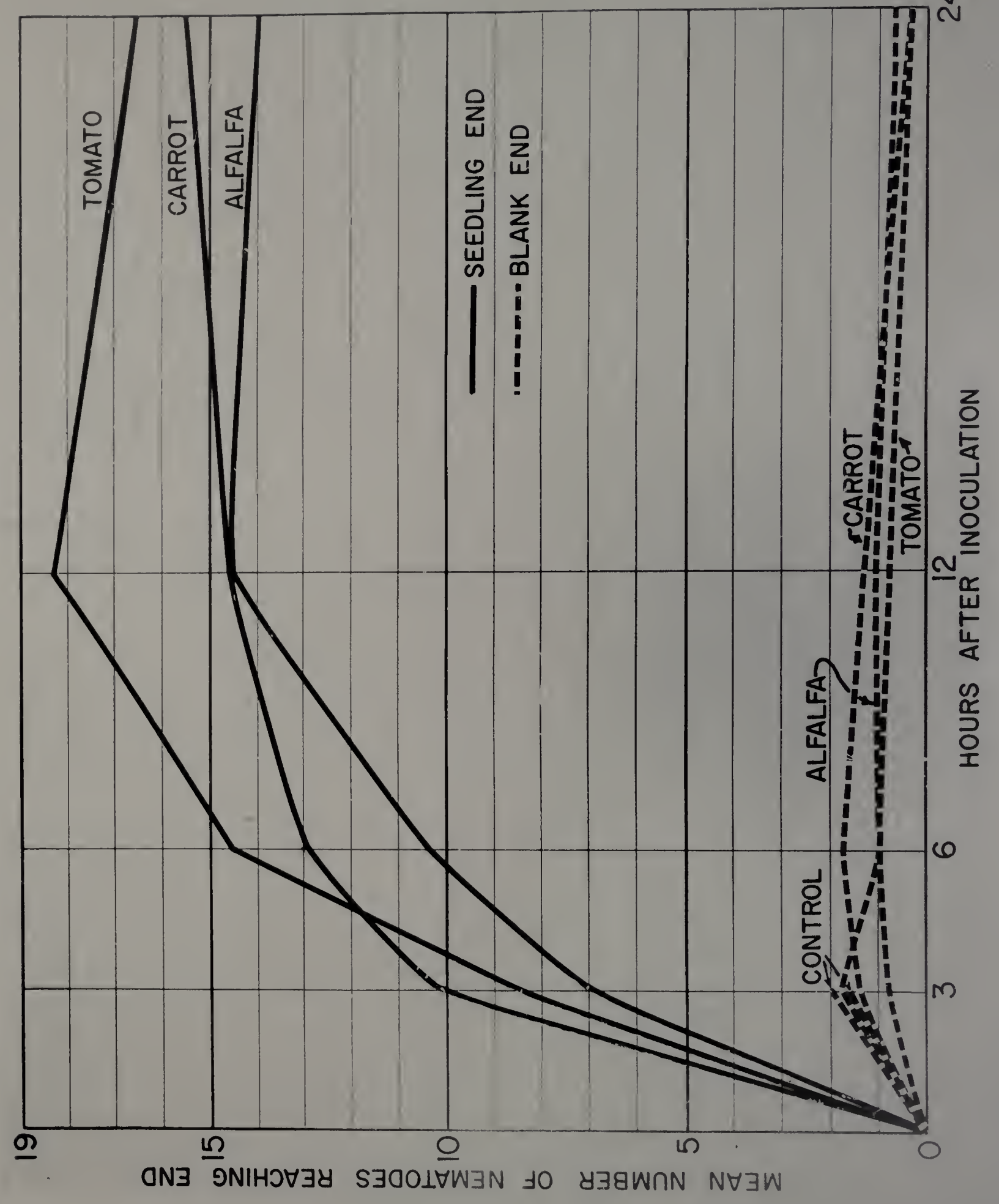

TOMATO
CARROT
ALFALFA
SEEDLING END
BLANK END
CARROT
TOMATO
ALFALFA
CONTROL
MEAN NUMBER OF NEMATODES REACHING END
0
5
10
15
19
3
6
12
24
HOURS AFTER INOCULATION

Figure 4. Migration of P. penetrans in agar to decapitated seedling roots of alfalfa, carrot, and tomato. Mean of 8 replications. Differences between plant and blank ends of chambers at the end of 3 hours were as follows: alfalfa chambers - not significant; carrot chambers - *, L.S.D. .05 = .267; tomato chambers - **, L.S.D. .05 = .225. The 3 and 6 hour data on migration to the plant end of whole seedling root chambers as presented in Fig. 3 is included in this figure for a comparison of these roots with decapitated seedling roots in regards to attraction. Data on migration to the blank end of chambers in both of these tests was essentially the same, thus only the data for decapitated seedling roots is presented here.

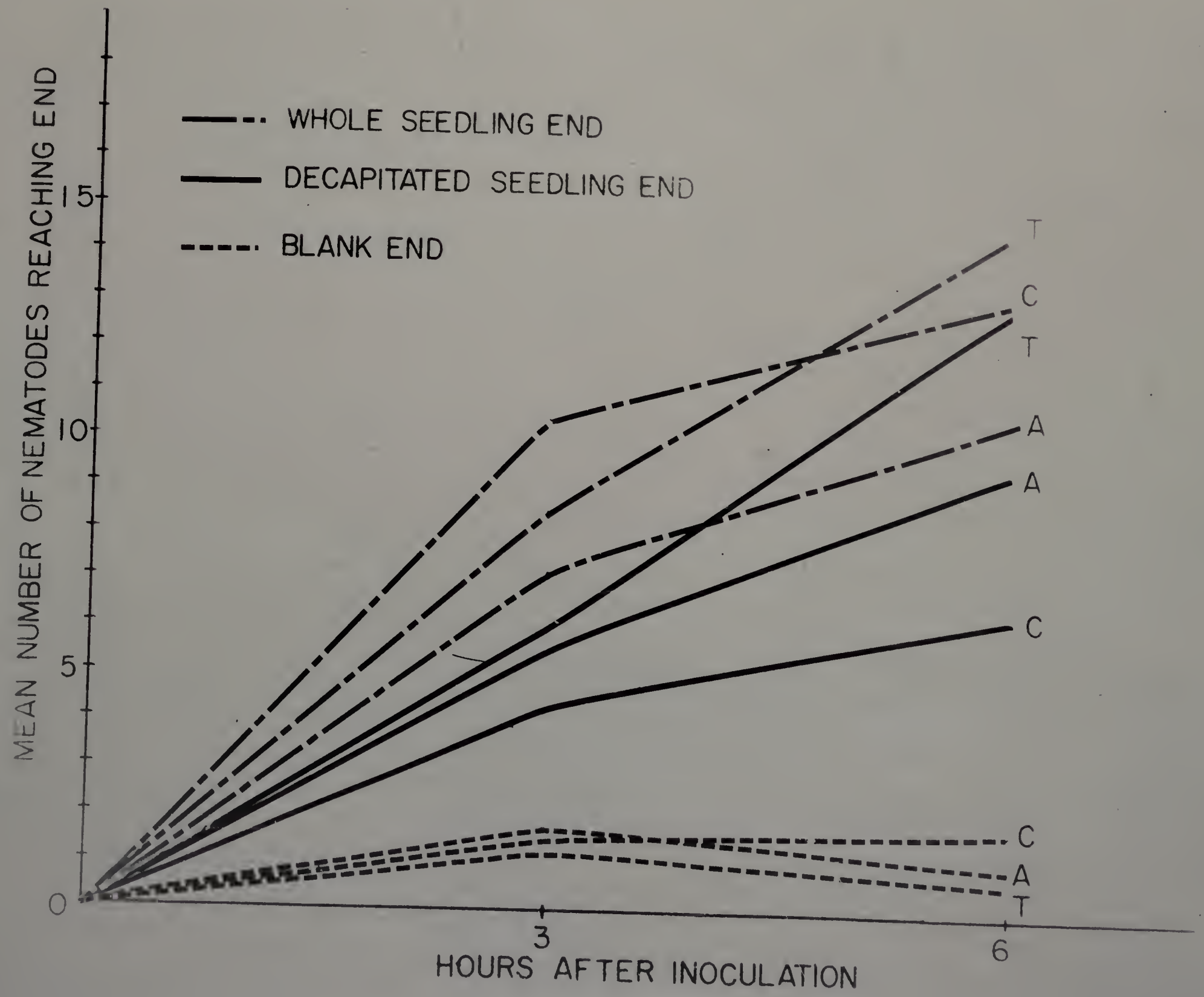

WHOLE SEEDLING END
DECAPITATED SEEDLING END
BLANK END
MEAN NUMBER OF NEMATODES REACHING END
0
5
10
15
3
6
HOURS AFTER INOCULATION
T
C
T
A
A
C
C
A
T

TABLE II. P. penetrans migration at the end of 3 hours in 1% water agar under the influence of alfalfa, carrot, and tomato seedling roots. Conditions of the hosts are excised roots of mature plants, removal of seedling roots prior to inoculation with nematodes, and decapitated seedlings. A, C, and T = plant ends of chambers. BL = blank ends of chambers.

Condition	treat-ment	Replications I	II	III	IV	V	VI	VII	VIII	$\bar{x}$	Log + one $\bar{x}$	.05 L.S.D.
Excised Roots	A	3	1	0	3	2	4	6	9	3.5	.516	n.s.
	BL	3	3	0	3	1	2	4	3	2.4	.486	
	C	7	2	7	1	6	3	11	7	5.5	.752	n.s.
	BL	0	6	3	0	3	5	6	8	4.0	.578	
	T	2	1	3	2	8	3	11	12	5.3	.701	n.s.
	BL	3	3	4	3	1	5	5	2	3.3	.605	
Removal of Roots	A	2	7	3	0					3.0	.496	n.s.
	BL	0	0	2	0					0.5	.119	
	C	1	4	4	1					2.5	.500	n.s.
	BL	0	0	1	3					1.0	.226	
	T	4	2	0	6					3.0	.505	n.s.
	BL	2	2	5	1					2.5	.508	
Decapitated seedlings	A	3	6	14	15	0	2	1	3	5.5	.651	n.s.
	BL	0	1	4	2	2	1	2	1	1.6	.379	
	C	4	7	5	5	0	4	3	6	4.3	.663	.267
	BL	0	2	2	2	1	0	2	1	1.3	.314	
	T	9	5	4	3	6	7	4	10	6.0	.821	.225
	BL	3	1	1	0	1	0	0	3	1.1	.263	

was less rapid than that of whole roots, and that the numbers of nematodes feeding on decapitated seedling roots were smaller, suggesting that less optimum conditions for root attractant production existed in the decapitated seedling roots. At the end of 6 hours, there were still fewer nematodes in the plant ends of decapitated seedling chambers as compared to the same ends of whole root chambers, but accumulation at the decapitated root surfaces was occurring (Table III).

C. Attraction to excised apical sections of mature host roots in agar:

Based on the criteria above, no attraction of nematodes to excised apical sections of roots of mature plants was found (Table II). Accumulation of nematodes around these roots is evident by 6 hours (Fig. 5, Table III). This evidence does not rule out the possibility that some attraction by mature roots occurs. In B. of this investigation it was found that decapitated young seedlings were not as attractive as whole ones with tops. No linear growth of the mature root sections was observed after excision. It appeared from observations of whole roots in the seedling stage that those exhibiting rapid linear growth were most attractive. Further investigations utilizing various zones of mature roots under conditions such that the roots remain attached to the living plant would do much to clarify this aspect of root attraction.

D. Attractiveness of areas in agar from which growing roots have been removed:

Areas where seedling hosts had been growing for 24 hours previous to inoculation did not meet the criteria for attraction at 3 hours (Fig. 6). The data do suggest, however, that attraction may occur and had a greater number of replications been used a statistical difference might have resulted (Table II).

TABLE III. P. penetrans migration at the end of 6 hours in 1% water agar under the influence of alfalfa, carrot, and tomato seedling roots. Conditions of the hosts are excised roots of mature plants, removal of seedling roots prior to inoculation with nematodes, and decapitated seedlings. A, C, and T = plant ends of chambers. BL = blank ends of chambers. Results are at least partly due to accumulation of nematodes at the root surface as random search of the entire chamber could have occurred during this time lapse.

Condition	treat-ment	Replications I	II	III	IV	V	VI	VII	VIII	$\bar{x}$	Log + one $\bar{x}$	.05 L.S.D.
Excised Roots	A	4	2	7	2	5	2	14	8	5.5	.743	n.s.
	BL	3	0	3	2	3	8	3	3	3.1	.555	
	C	5	1	7	4	8	6	10	8	6.1	.809	n.s.
	BL	5	5	3	1	4	4	1	5	3.5	.617	
	T	1	5	5	5	9	3	12	15	7.0	.819	n.s.
	BL	0	5	2	5	2	8	4	2	3.5	.580	
Removal of Roots	A	3	8	5	2					4.5	.703	.387
	BL	0	2	0	1					0.8	.195	
	C	7	11	3	4					6.3	.827	.338
	BL	1	0	0	1					0.5	.151	
	T	3	1	6	5					3.8	.632	n.s.
	BL	0	3	1	0					1.0	.226	
Decapitated seedlings	A	3	11	22	16	5	2	6	10	9.4		
	BL	0	0	2	1	1	0	1	2	0.9		
	C	7	8	9	7	2	4	4	9	6.3		
	BL	0	2	1	6	3	0	1	0	1.6		
	T	14	11	7	11	11	11	14	24	12.9		
	BL	0	0	0	1	3	0	1	0	0.6		

Figure 5. Migration of P. penetrans in agar to excised apical sections of mature host roots of alfalfa, carrot, and tomato. Mean of 8 replications. Differences between plant and blank ends of chambers at the end of 3 hours were not significant for any of the 3 hosts tested.

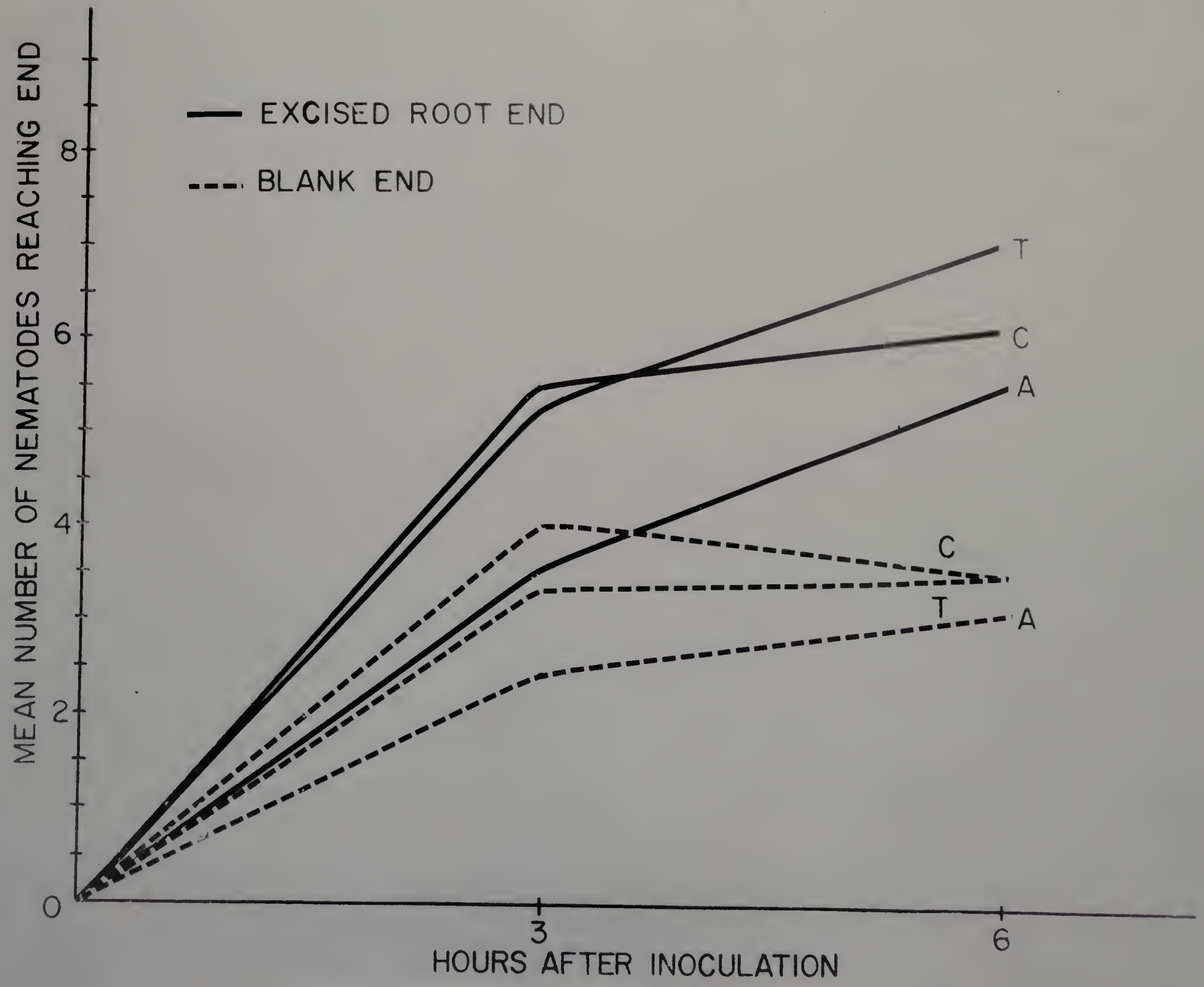
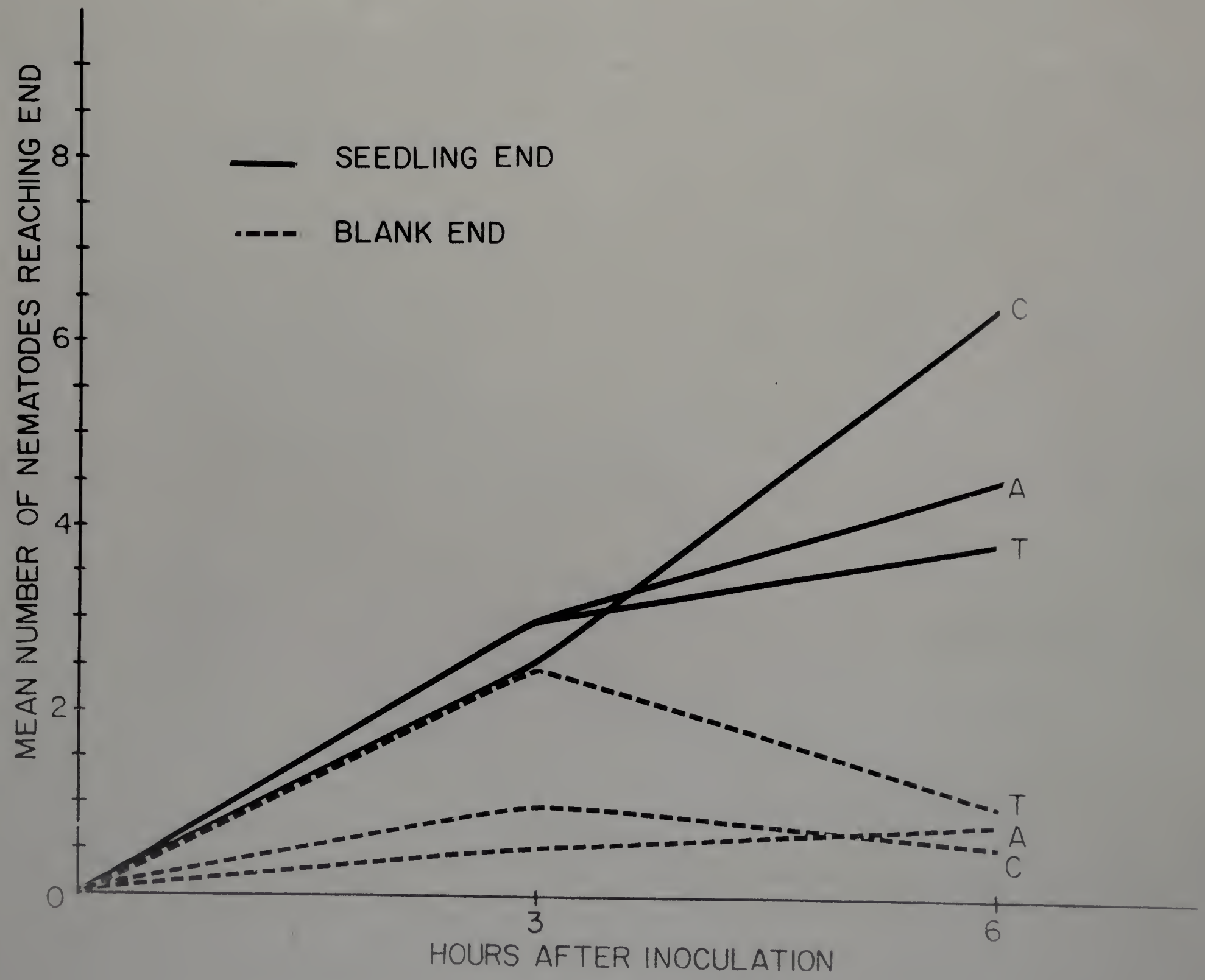

EXCISED ROOT END
BLANK END
MEAN NUMBER OF NEMATODES REACHING END
0
2
4
6
8
3
6
HOURS AFTER INOCULATION
T
C
A
C
T
A

Figure 6. Migration of P. penetrans in agar to areas from which growing seedling roots of alfalfa, carrot, and tomato have been removed. Mean of 4 replications. Differences between plant and blank ends of chambers at the end of 3 hours were not significant for any of the 3 hosts tested. At the end of 6 hours differences were as follows: alfalfa chambers - *, L.S.D. .05 = .387; carrot chambers - **, L.S.D. .05 = .338; tomato chambers - not significant.


MEAN NUMBER OF NEMATODES REACHING END
8
6
4
2
0
SEEDLING END
BLANK END
C
A
T
T
A
C
3
6
HOURS AFTER INOCULATION

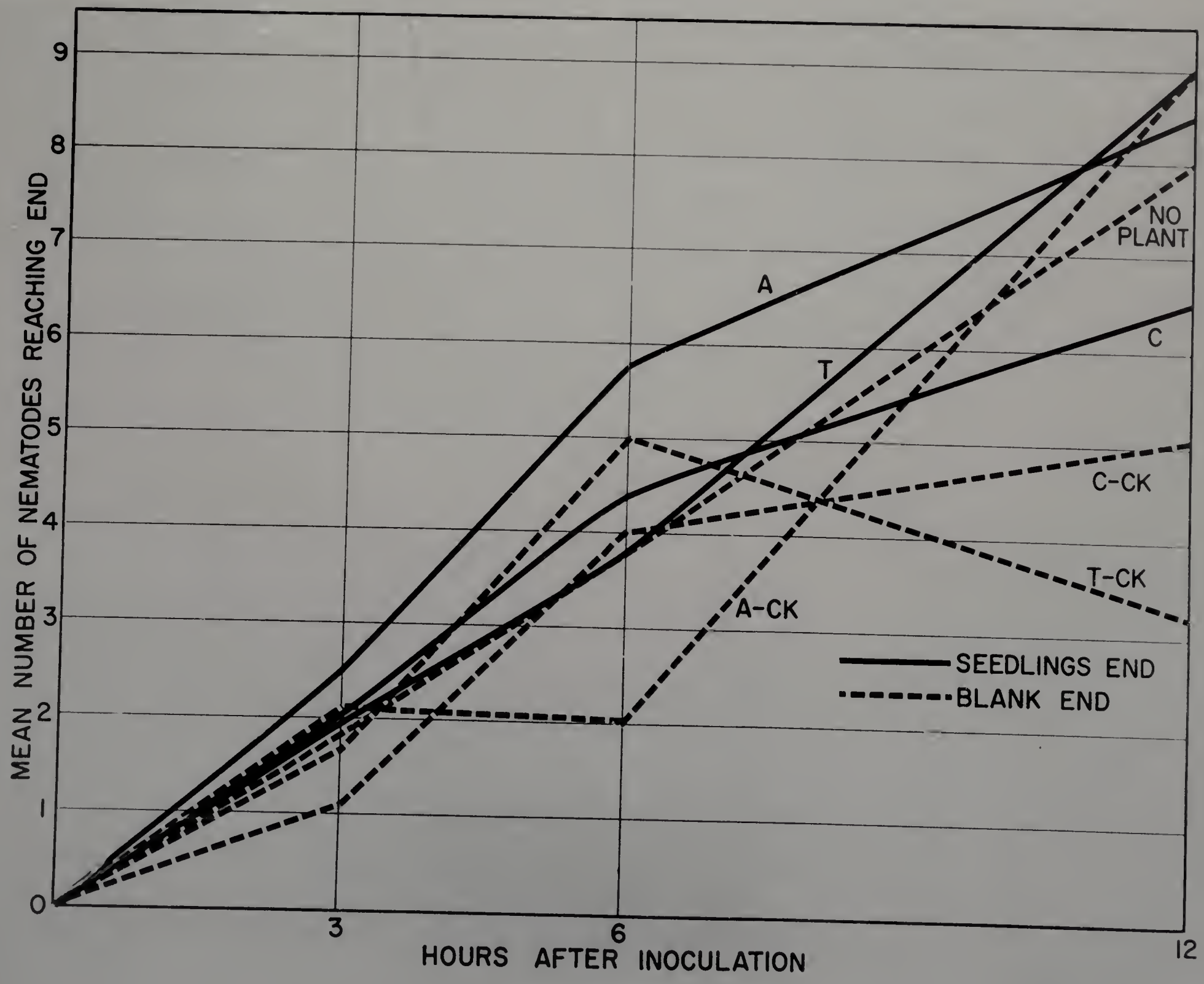
Removal of the host seedling root presumably removed any tangible food source (excepting that possibly the exudate itself was food) from the nematodes leaving only the root exudations, bacteria, and a few root cap cells which had sloughed off during growth. Feeding of nematodes on these cells was not observed. Therefore, it seemed reasonable to assume that if a greater number of nematodes were on the plant side in 6 hours, they were attracted there or accumulated because of some lingering attractant in the agar. At 6 hours the differences between plant and blank ends were significant in the case of alfalfa, highly significant for carrot, but not significant for tomato (Table III). Despite this failure of the tomato area to attract at 6 hours, the graphed data shows that the trend is similar to the other hosts. It is possible that the nature of the tomato attractant is such that it is rapidly lost from the agar, that its gradient is easily dissipated, or less attractant is produced. Also, contamination of the agar by fungi in some of the tomato chambers may have had an influence on attraction.

These results agree basically with Wallace's conclusion that certain nematodes are attracted to areas where roots have been growing in sand. These evidences strongly suggest that a root or bacterial exudation or both have attractant properties. Circumstances of this test favor root exudations as a cause since the seedlings and the agar were originally sterile. It is unlikely that there would be a uniform contamination of all the chambers with the same bacterium.

E. Nematode migration to seedling roots in sand:

Attraction of P. penetrans to alfalfa seedling roots in sand was found (Fig. 7). In 6 hours, the critical time lapse, highly significant differences

Figure 7. Migration of P. penetrans in quartz sand to seedling roots of alfalfa, carrot, and tomato and to blank ends of control chambers at 3 and 12 hours after inoculation of chambers with nematodes. Mean of at least 9 replications. Differences between plant and blank ends of chambers at the end of 3 hours were not significant for any of the 3 hosts tested. At the end of 6 hours, alfalfa chambers showed - **, L.S.D. .05 = .239; carrot and tomato differences were not significant. At the end of 12 hours, tomato chambers showed - **, L.S.D. .05 = .242; carrot and alfalfa differences were not significant. Differences between blank ends of control chambers and blank ends of seedling chambers at 3 and 12 hours after inoculation with nematodes were not significant.


MEAN NUMBER OF NEMATODES REACHING END
0
1
2
3
4
5
6
7
8
9
3
6
12
HOURS AFTER INOCULATION
A
T
C
NO PLANT
C-CK
T-CK
A-CK
SEEDLINGS END
BLANK END

were recorded between plant and blank ends (Table IV). Tomato and carrot tests did not exhibit this effect.

In tests comparing results from ends of chambers after 12 hours, tomato trials exhibited a highly significant difference, but this may be partly due to accumulation. Alfalfa and carrot chambers did not show differences.

The nearly straight line relationship established by the control chambers having no plants is of interest in this investigation. This line indicates that when no host is present, nearly equal numbers of nematodes move out toward both ends of chambers. If this is accepted as a comparison, the stimulatory effect of adding the seedling becomes apparent as seen by the alfalfa results at 6 hours.

The failure of the carrot and tomato seedlings to attract nematodes in 6 hours may be attributed to the distance of the roots from the inoculation site or to a slower rate of diffusion of the exudate in sand. The same distance of seedling from inoculation site and the same chamber pre-conditioning time was used for sand and agar. It is very likely that the sand was a less uniform media than agar, and had a higher osmotic pressure. Both of these factors may have influenced exudate movement.

Lack of accumulation by 12 hours in carrot and alfalfa plant ends of chambers may possibly be due to a feature of the mass of the population inoculated. Presumably, at 6 hours only the explorers have moved measureable distances from the inoculation site, and those reaching the plant end have stayed there. In 12 hours, however, the mass of the population, which may or may not move in response to a root exudate (it is unknown whether all life stages of P. penetrans are affected by root exudates or whether manipulation alters the ability to detect exudates) has begun to

TABLE IV. P. penetrans migration in quartz sand under the influence of alfalfa, carrot, and tomato seedlings, at 3, 6, and 12 hours after inoculation of chambers with nematodes. A, C, and T = plant ends of chambers. BL = blank ends of chambers.

Time	treat-ment	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	$\bar{x}$	Log + one $\bar{x}$	.05 L.S.D.
	A	1	2	12	3	0	1	4	0	1	1			2.5		
	BL	4	1	8	0	3	2	1	0	1	1			2.1		n.s.
3	C	5	5	1	6	0	1	0	0	1	1			2.0		
HOURS	BL	0	3	2	3	1	0	0	1	2	0			1.2		n.s.
	T	7	1	7	-	1	0	1	0	0	0			1.9		
	BL	0	1	6	-	2	0	1	5	0	0			1.7		n.s.
	A	12	5	13	2	12	3	3	4	3	10	2	1	5.8	.750	
	BL	1	0	2	5	2	0	1	1	1	3	5	2	2.0	.399	.239
6	C	4	5	4	11	1	1	2	8	2	6	6	3	4.4		
HOURS	BL	4	4	3	4	11	6	3	1	3	5	2	2	4.0		n.s.
	T	3	7	1	2	6	6	2	3	2	9	3	2	3.8		
	BL	3	5	7	3	5	0	5	4	3	10	13	3	5.0		n.s.
	A	12	36	5	3	0	3	9	0					8.5	.708	
	BL	5	6	32	16	7	1	4	1					9.0	.822	n.s.
12	C	12	10	4	7	3	4	7	5					6.5	.842	
HOURS	BL	7	5	8	4	9	3	2	3					5.1	.752	n.s.
	T	13	13	16	3	5	9	8	4					9.0	.944	
	BL	2	5	1	7	4	1	2	4					3.2	.579	.242

move from the inoculation site. A random migration to blank and plant plant ends by large numbers of individuals could, conceivably increase the variation or error and thereby throw off the effect of the explorers, resulting in the failure of a statistical test to detect a difference. In addition, possibly some nematodes had penetrated the roots at 12 hours and were not counted, yet were present.

It is doubtful that a chemokinesis is involved (at least not more than a few mm from the roots) in these tests. Using the controls as examples of movement occurring without host influence, it was found that no significant differences exist between ends of these chambers and blank ends of plant chambers.

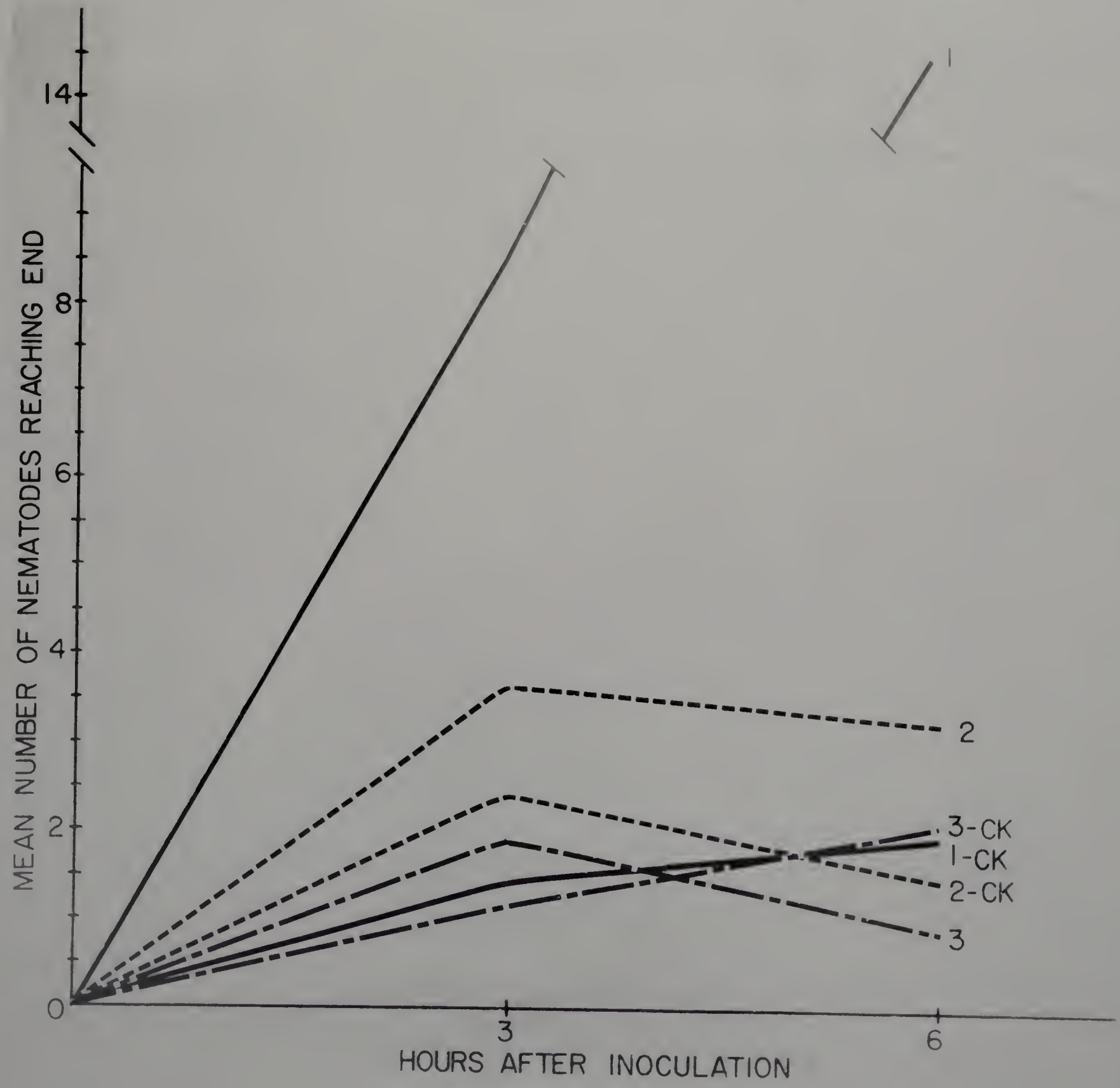
F. Distance over which attraction to tomato seedlings is effective:

Attraction of tomato seedling roots in agar could not be demonstrated when distances greater than 12.5 mm from the site of inoculation were used. Data obtained in tests (Fig. 8) at distances of 44.5 mm and 69.5 mm from the site of inoculation yielded differences between plant and blank ends that were not significant.

The failure of nematodes to be attracted to roots at distances greater than 12.5 mm from the site of inoculation or in time periods of less than 24 hours of chamber pre-conditioning by seedlings suggests that the attractant is a diffusable substance. The speed of diffusion of materials through the agar was not calculated, but considering the nature of this medium, it apparently was not a rapid process.

The graphed data for the 44.5 mm site shows nearly twice the number of nematodes going to the plant end as compared with the number going to the blank end, while the data for the 69.5 mm site shows nearly equal

Figure 8. Migration of P. penetrans in agar to seedling roots of tomato placed at 3 distances away from the inoculation site. Distance code: 1 = 12.5 mm; 2 = 44.5 mm; 3 = 69.5 mm. Blank ends of chambers are identified by the proper distance code plus CK.
Mean of 5 replications.


MEAN NUMBER OF NEMATODES REACHING END
0
2
4
6
8
14
3
6
HOURS AFTER INOCULATION
1
2
3-CK
1-CK
2-CK
3

numbers going to each end of the chambers. This could mean that the seedlings at the 44.5 mm site produced a slight attractant effect, but not enough to produce a statistical difference between ends.

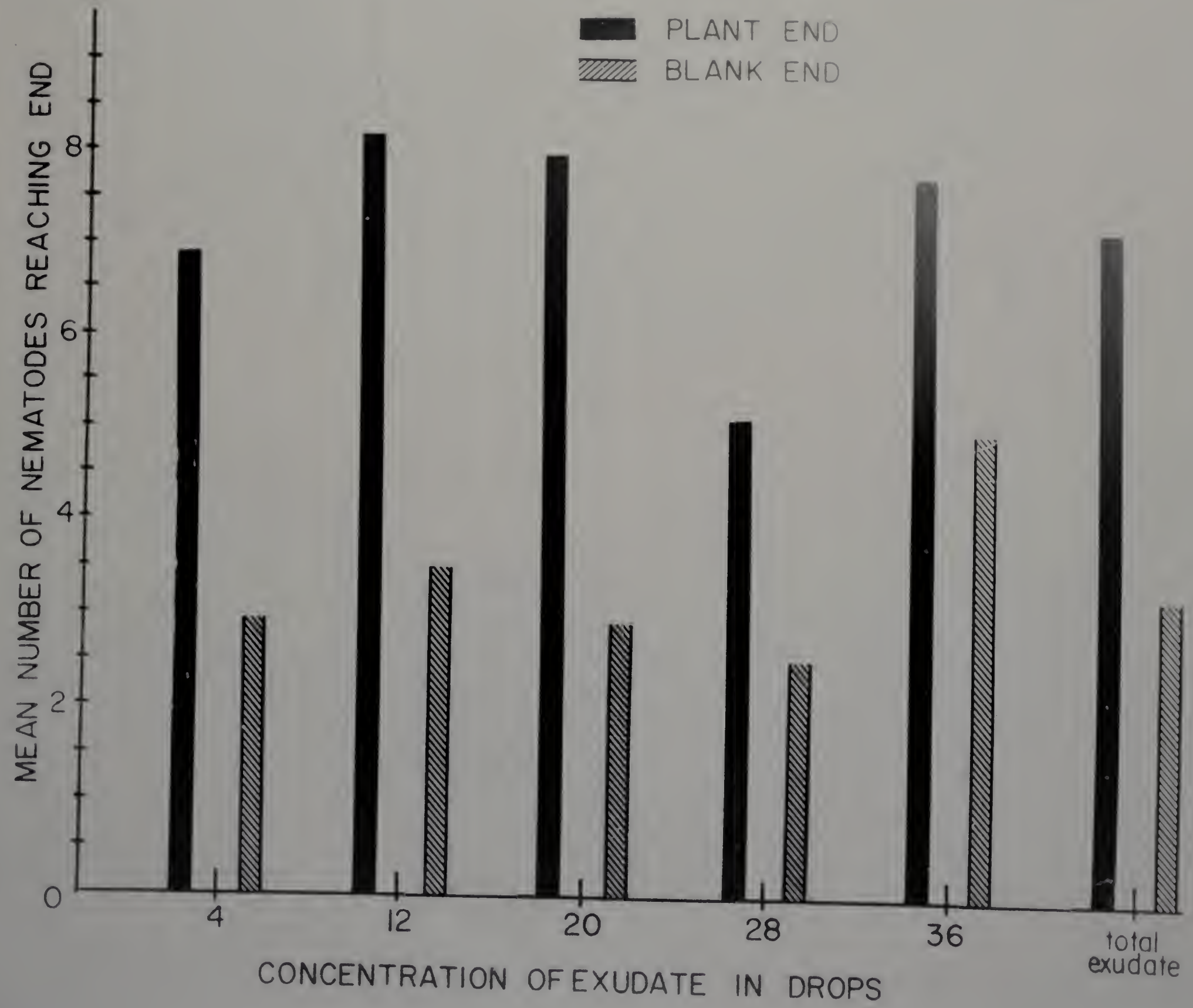
In summary, if a diffusable substance is responsible it is possible that this substance either moves so slowly that it does not reach the inoculation site from distances greater than 12.5 mm after 24 hours pre-conditioning, or at distances such as these the substance becomes lost or altered in some way so that attractant properties are nil. It may be that failure of attraction is due to lack of a sufficient concentration building up, but results obtained with exudate alone suggest that the concentration required for attraction is quite low.

G. Effect of tomato root exudate on nematode migration:

Results obtained (Fig. 9) at 6 hours after inoculation of chambers containing tomato exudate in paper disks at five different concentrations showed significant differences between "plant" (exudate) and blank ends at the 4 and 28 drop concentration and a highly significant difference at the 12 drop concentration. By reason of the high numbers of nematodes in the disks at 6 hours, it appears that these disks are much more easily penetrated by the nematodes than are seedling roots. Instead of building up on the surface, as with plants, they pass into the paper, the probable effect of which is an inaccuracy of the counts made at the prescribed time lapses.

A valid comparison of the response is to test the number of nematodes in plant disks plus those in the plant end against those in the blank disk plus blank end at the end of 6 hours. It is acknowledged that accumulation of nematodes could have occurred by this time, but, as in the tests

Figure 9. Migration of P. penetrans in agar to tomato seedling root exudate contained in paper disks at 6 hours after inoculation of chambers with nematodes. Mean of 9 replications. Differences between "plant" (exudate) and blank ends of chambers were as follows: 4 drop concentration chambers - *, L.S.D. .05 = .344; 12 drop concentration chambers - **, L.S.D. .05 = .182; 28 drop concentration chambers - *, L.S.D. .05 = .190. Other concentrations showed no significant differences. The overall difference between blank and "plant" ends was highly significant. Statistical tests were based on 3 replications.


PLANT END
BLANK END
MEAN NUMBER OF NEMATODES REACHING END
0
2
4
6
8
4
12
20
28
36
total exudate
CONCENTRATION OF EXUDATE IN DROPS

involving removal of the seedlings, the only possible food source present is the exudate alone.

The overall difference between plant and blank counts was highly significant. The reason for some of the individual concentrations not showing differences is possibly the lack of sufficient replication.

These results strongly suggest that root exudate does affect nematode migration. With further refinement of techniques and investigation of the attractant involved, the nature and importance of a specific attractant or compound may be found.

H. Observations of the root areas producing attraction and determination of the point of nematode entry:

Information relating to this objective was obtained by observation only (Fig. 10). The experiment was not designed to statistically evaluate any feeding preference of the nematode for one area of a root over another.

In the study with uninjured roots, feeding and quiescence occurred in all regions of the root below the agar surface. A tendency to accumulate at the root zone of elongation or just behind the root cap, such as has been found with several other species (Christie, 1959), was rarely observed when whole seedlings were used. Migration from the inoculation site to the plant end was not directed toward any particular root area, but usually, the nematodes stayed in the area where they first contacted roots. Often, however, nematodes would pass over or under the root and stop when contacting a section of root either beyond or lateral to the point closest to the center of the chamber.

In the study with injured roots, accumulation occurred around cut ends of tomato root pieces. In preliminary experiments with Flame marigold (Tagetes patula L.), whose roots tend to branch soon after germination,

Figure 10. Activity of P. penetrans upon the surface of and within carrot seedling roots which have germinated for 6 days.

A. Accumulation and feeding of nematodes on root surface approximately 3-6 hours after inoculation of chambers. X88.

B. Section of root showing entry hole (arrow) made by nematodes and discoloration caused by feeding. X88.

C. Entry hole (arrow) made by nematodes. X376.

D. Entry hole (arrow) showing tail of nematode protruding through the opening. X849.

E. Entry hole (arrow) showing nematode tail just passing through the opening. X849.

F. Nematode inside root attacking the end wall of a cell. Note bulging and distortion of end wall (arrow). X849.

A

B

C

D

E

F

accumulation occurred around the disturbed areas of primary roots through which secondary roots were penetrating. Accumulation in the zone of elongation and just behind the root cap was noted for excised roots and decapitated seedlings of all three hosts. A less pronounced accumulation in other root areas did occur, however, in these tests.

Penetration occurred by two methods. The first and perhaps most common method consisted of one or several nematodes feeding constantly in an area of root for 3-9 hours. During this time epidermal cells were broken down and a small hole was formed (Fig. 10). Discoloration of the epidermis and cortex became evident shortly after the epidermis was punctured and the nematode or nematodes passed into the root. Other nematodes apparently passed through this hole into the root. Often, nearly a dozen nematodes were visible in the surrounding root tissue beneath or near one of these punctures.

The second method observed was direct entry into roots through disturbed root tissue or cut ends of roots. Alfalfa and marigold roots were penetrated in the area of root disturbed by outgrowing secondary roots. Entry through cut ends of tomato root pieces was observed.

Discoloration and entry of roots was evident shortly after the first nematode reached the root surface. Carrot root tissue was discolored within 3-4 hours and tomato and alfalfa root tissue after 8-9 hours of proximate nematode association (presumably feeding). Entry into the roots of all three hosts began about 5-12 hours after the first nematode had reached the roots.

In one trial a carrot seedling suffered extremely heavy attack by 40 nematodes. At 28.5 hours after inoculation the aerial parts of this

plant wilted and a slight chlorosis of the leaves was noted. Discoloration of the roots was extensive. A less intensively injured carrot seedling of the same age was green, upright growing, vigorous, and exhibited no abnormalties.

VI CONCLUSIONS

A chemotaxis[1] was responsible for root finding by nematodes in this investigation. P. penetrans was attracted by roots of carrot, alfalfa, and tomato seedlings over distances of at least 12.5 mm in agar. Evidence of this was seen in the highly significant differences in numbers of nematodes between plant and blank ends of polystyrene chambers after 3 hours. Further evidence was the accumulation of nematodes in areas of agar where the seedlings had been growing (but had been removed prior to inoculation with nematodes), and accumulation in and around paper disks containing tomato seedling root exudate. In sand, attraction of nematodes to alfalfa seedling roots occurred. Although not statistically significant, there was a trend for the tomato and carrot to show attraction similar to alfalfa in sand.

The attractant from seedling roots was a diffusable substance produced by the plant. Attraction occurred only after seedlings had pre-conditioned chambers for 24 hours, and when roots were placed 12.5 mm or closer to the nematodes. Since sterile conditions were maintained in initial phases of the tests, it is improbable that a uniform contamination of all the chambers by bacteria occurred. Nematode accumulation and entry of roots at cut ends, and feeding wounds made by other individuals where, presumably, chemicals were being released in large amounts adds supporting evidence.

Attraction was most pronounced when the seedlings were actively growing and linear root growth was rapid. Decapitated seedlings and excised roots of mature plants either did not produce attraction or had a less

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[1] Terminology from Fraenkel and Gunn (1940).

pronounced effect on the nematodes, suggesting less optimum conditions for attractant production. Since P. penetrans was attracted to and fed on many areas of intact seedling roots, it was possible that all areas of the root produced the attractant.

P. penetrans was stimulated by attractant concentrations of 4 or more drops per unit area of filter paper disks. In comparisons of blank and exudate containing disks, more nematodes accumulated in and around the exudate disks when these concentrations were used.

A chemokinesis was not responsible for root finding, except possibly in areas a few mm from the root. Significant differences between blank ends of control chambers and blank ends of seedling chambers were not found in agar at the end of 3 hours or in quartz sand at the end of 3 and 12 hours.

Differences in degree of attraction between sand and agar were possibly due to different diffusion rates of the exudate in the two media. Since the sand system was not uniform (solid and liquid) and probably had a higher osmotic pressure than agar, diffusion of the exudate was slower, resulting in a failure, in the cases of tomato and carrot tests in sand at the end of 6 hours, of the nematodes at the inoculation site to be stimulated. Root exudate obtained from tomato seedlings growing in a sand culture proved attractive in tests by itself, suggesting that the exudate was not decomposed or its attractant nature altered by the sand.

Nematode penetration of roots was through small holes punched in the root epidermis by a small number of nematodes, through which large numbers of individuals passed, and through cut ends of roots and disturbed root tissue. Observations of discoloration and possible tyloses in attacked seedling roots suggest that, in addition to wounding, the nematodes

produce an antagonistic effect, a condition reported by other investigators (Pitcher, Patrick, and Mountain, 1960) on other plant species.

A comparison of the means of alfalfa, carrot, and tomato in the various tests (see Tables II, III, and IV) did not disclose any indications that one host was more attractive than another. Since hosts were not tested against each other in the same chamber (the choice was either nothing-blank or a single host), there was no basis for statistically testing the attraction by hosts against each other.

VII SUMMARY

Attraction of P. penetrans to alfalfa, carrot, and tomato seedlings roots was demonstrated using small polystyrene chambers containing one per cent water agar over short time periods during which nematode accumulation by random movement could not occur. The factors of size of the chamber in which the nematodes and roots were placed and rate of movement of the fastest nematode in the medium without the root influence were considered.

Nematodes were attracted to alfalfa seedlings in sand moistened to field capacity. Similar polystyrene chambers were utilized in these tests and attraction was measured considering the factors noted above.

The fastest rate of migration of P. penetrans in agar was 10 mm per hour. In sand the fastest rate was 4-5 mm per hour. This investigation was mainly concerned with the first nematodes reaching the stimulus.

Standardized conditions were maintained as strictly as possible throughout the investigation. All tests were conducted at 26 - 27°C.

Utilizing the basic technique mentioned above for agar, the following conclusions were reached: (1) excised seedling roots in agar were attractive to nematodes, although to a lesser degree than whole, unexcised roots; (2) at the end of 6 hours nematodes accumulated in areas of the agar where seedling roots had been growing for 24 hours prior to inoculation with nematodes; (3) nematode accumulation at mature host excised root apices in agar was noted at the end of 6 hours; (4) the attractant from tomato seedlings appeared to extend more than 12.5 mm but less than 44.5 mm from the root in agar; (5) tomato root exudate contained in filter paper

disks caused accumulation at the end of 6 hours.

In both sand and agar no differences between ends of control chambers and blank ends of plant chambers was detected, suggesting that a chemokinesis was not responsible for the nematode response.

Observations of nematodes attacking all regions of host roots in agar suggested that attraction to all areas of the roots of whole seedlings occurred. Discoloration of root tissue was evident in 3-4 hours after the first nematodes reached the roots on carrot and 8-9 hours on tomato and alfalfa. Penetration was evident at the end of 5-12 hours after the first nematodes reached the roots. Entry of roots occurred by passage of several or many nematodes through the feeding wounds made by single nematodes, through cut ends of roots, and through disturbed areas of roots.

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IX APPENDIX

A. Statistical methods used in testing data:

The analysis of variance technique as presented by Steel and Torrie was used to test differences between plant and blank ends of chambers[1]. Modifications of this technique were made only insofar as the actual observations underwent logarithmic transformation[2]. To clarify the use of this method, the following example is given:

1) Carrot seedling in agar - 3 hours after inoculation.

Carrot end			Blank end		
actual obs.	add 1	logarithm	actual obs.	edd 1	logarithm
8	9	.954	1	2	.301
6	7	.845	1	2	.301
8	9	.954	0	1	.000
5	6	.778	3	4	.602
10	11	1.041	0	1	.000
25	26	1.415	0	1	.000
3	4	.602	1	2	.301
17	18	1.255	1	2	.301
		7.844			1.806

Sum of observations = 9.650
Correction term = 4.049
Total sum of squares = 5.601
Group sum of squares = 4.050
Individual sum of squares = 1.551

Analysis of Variance Table

Source	Degrees of freedom	Mean square
Group	1	4.050
Individual	14	.111
Total	15	

carrot $\bar{x}$ = .981, blank $\bar{x}$ = .226, F = 36.486 **, L.S.D. = .357

$$\text{L.S.D. } .05 = t_{.05}\left(\sqrt{\frac{2s^2}{n}}\right) = 2.145\ (\sqrt{.1666}) = .357$$

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1. Steel, R.G.D. and J.H. Torrie. (1960). Principles and Procedures of Statistics. New York. McGraw-Hill, 481 pp. (See pp. 101-107)
2. Steel and Torrie, p. 157.

The statistical procedure used to test differences between means of the blank ends of chambers with plants and the blank ends of control chambers is considered by Steel and Torrie in their discussion of unpaired observations and unequal variances[3] . Transformation of actual observations to logarithms occurred in these tests, also. The following example illustrates this procedure:

2) Comparison of blank ends of control chambers and blank ends of plant chambers after 12 hours in sand.

Control blank			Alfalfa blank			Carrot blank			Tomato blank		
actual obs.	add 1	log	actual obs.	add 1	log	actual obs.	add 1	log	actual obs.	add 1	log
10	11	1.041	5	6	.778	7	8	.903	2	3	.477
3	4	.602	6	7	.845	5	6	.778	5	6	.778
3	4	.602	32	33	1.518	8	9	.954	1	2	.301
8	9	.954	16	17	1.230	4	5	.699	7	8	.903
3	4	.602	7	8	.930	9	10	1.000	4	5	.699
1	3	.301	1	2	.301	3	4	.602	1	2	.301
2	3	.477	4	5	.699	2	3	.477	2	3	.477
6	7	.845	1	2	.301	3	4	.602	4	5	.699
3	4	.602									
20	21	1.322	εX =	6.602		εX =	6.015		εX =	4.635	
16	17	1.230									
4	5	.699	εx^2 =	5.944		εx^2 =	4.772		εx^2 =	3.034	
31	32	1.505									
6	7	.845									
6	7	.845									
5	6	.778									
εX =	13.250										
εx^2 =	12.523										

	Controls	Plants
εX	13.250	17.252
εx^2	12.523	14.477
$\bar{x}$	.828	.719
εx_1^2	1.550	εx_2^2 2.076
$s_1^2 = \frac{\varepsilon x_1^2}{n_1 - 1}$	1.033	$s_2^2 = \frac{\varepsilon x_2^2}{n_2 - 1}$ .903

3. Steel and Torrie, pp. 81-83.

$$\bar{sd} = \sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}} = \sqrt{.1021} = .3199$$

$$t' = \frac{\bar{d}}{\bar{sd}} = \frac{.109}{.3199} = .341 \text{ not significant}$$

B. Scientific names and synonomy of nematode species mentioned in this investigation:

The order of the following names is based on their appearance in the manuscript. The present scientific name is given for those combinations which have been reduced to synonomy.

Present Scientific Name	Synonym Used in Manuscript
Meloidogyne Göldi, 1887.	Heterodera marioni (Cornu, 1879) Goodey, 1932.
* Pratylenchus pratensis (DeMan, 1880) Filipjev, 1936.	Anguillulina pratensis (DeMan, 1880) Goffart, 1929.
Rotylenchus Filipjev, 1936.	
Meloidogyne hapla Chitwood, 1949.	
Heterodera rostochiensis Wollenweber, 1923.	
* Pratylenchus penetrans (Cobb, 1917) Filipjev & Shuurmans Stekhoven, 1941.	Anguillulina pratensis in Goodey, 1932 and 1933; W. Schneider, 1939.
	Pratylenchus pratensis in Filipjev & Schuurmans Stekhoven, 1941; Goodey, 1951.
Heterodera schachtii Schmidt, 1871.	
* Pratylenchus brachyurus (Godfrey, 1929) Goodey, 1951.	Pratylenchus pratensis in Thorne, 1940.
* Pratylenchus zeae Graham, 1951.	
Meloidogyne incognita (Kofoid & White, 1919) Chitwood, 1949.	

Meloidogyne javanica (Treub, 1885) Chitwood, 1949.

Meloidogyne incognita acrita Chitwood, 1949.

Ditylenchus dipsaci (Kuhn, 1857) Filipjev, 1936.

** Panagrellus redivivus (Linn., 1767) Goodey, 1945.

** Turbatrix aceti (Müller, 1873) Peters, 1927.

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All other names from Christie, J.R. (1959). Plant Nematodes. Gainesville. Agricultural Experiment Station, University of Florida. 256 pp.

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